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Recent researches on prebiotics for gut health in Thailand

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ABSTRACT

Background: In the food industries, several oligosaccharides have received increasing attention as key components for functional foods and nutraceutical products. Prebiotics are non-digestible oligosaccharides which have been shown to have properties that can modulate gastrointestinal problems and improve gut health and well-being. Recent researches much pay attention to find alternative sources, improve specific properties and proof on health benefits of these prebiotics.

Methods: This is the summary of research works have been done by our research group on prebiotics and gut health in Thailand. These works aimed to study sources of prebiotics from fruits and vegetables in Thailand, production by enzymatic synthesis of prebiotics, purification by microbial fermentation and membrane technology and applications of the prebiotics in nutraceuticals and functional foods.

Results: Among the 30 parts of 14 plants, six appear to have the highest potential for commercialization based on extract yield and the amount and type of indigestible oligosaccharides. These include dragon fruit, palm flesh, palm embryo, jackfruit flesh, jackfruit seed, and okra pod. At least three of them, dragon fruit, jackfruit flesh and seed, were confirmed on their prebiotic property by selectively *in vitro* colonic microflora fermentation in an artificial colon system. Among 52 LAB isolates for production of GOS, BFP32 showed highest intracellular β-galactosidase activity and GOS yield. It was identified as *Lactobacillus pentosus* var. *plantarum* by 16S rDNA sequencing. Composition of GOS consisted of oligosaccharides with having DP of 3, 4 and 5. A mixture of GOS was purified successful by sequential bacterial and yeast fermentation whereas nanofiltration could be used for partial purification. Prebiotic index (PI) of the GOS produced was1.19 in batch culture. A crude extract from tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) had transfructosylating activity for biosynthesis of FOS from sucrose. Optimal conditions for production of FOS were 0.26 U FTase, incubated with 0.46 M sucrose as substrate at pH 5.4 and 35ºC for 144 h. A maximum yield of scFOS (DP<5) was obtained (54.46%). The scFOS showed prebiotic property with PI of 1.29 in batch culture. Four formulas of canned tuna in spring water and tuna in mayonnaise and pouched tuna in salad cream and tuna in thousand island cream with added inulin were developed for commercial production. An addition of 5 % inulin for tuna in spring water and 7 % inulin for tuna in mayonnaise, tuna in thousand island and tuna in salad cream are recommended. The highest prebiotic index (PI) scores of tuna in spring water and tuna in salad cream added 5% inulin were 1.82 and 0.93, respectively in three-stage continuous culture. In clinical study of tuna products, it was found that 5% inulin addition helps on improve of bowel regularity. By-product from rubber wood sawdust could be used as alternative source of XOS. Among eight edible commercial mushrooms, *Schizophyllum commune*had highest total β-glucan content (59.87% dry basis).

Conclusions: Research on prebiotics in Thailand had two mainly approaches including by extraction from natural occurring in fruits and vegetables and by enzymatic approach using hydrolysis and transferase activities from agricultural by-products and low cost raw materials. Prebiotic researches are covered *in vitro*, *in vivo* in rat and being investigated in clinical study related to gut health functional and immunity.

Keywords: Prebiotic, Functional carbohydrate, Gut health, Fecal fermentation, Clinical study

BACKGROUND

Prebiotic is emerging functional food ingredient. It is classified as soluble dietary fiber that mostly composes of oligosaccharides which can promote the growth of beneficial microorganisms or probiotics. Health claim for consumption of prebiotic is improve on gut health function and nutrient claim is as source of dietary fiber [1]. Prebiotic is one of the prevention alternatives. Prebiotics are nonviable food components which escape digestion in the upper gut, are fermented by probiotics or beneficial bacteria. Prebiotics are distinguished from other dietary fibers because of it has special property on selective fermentation by bifidobacteria/ lactobacilli within the gut microflora [2]. In addition, prebiotic has been linked to improved colon health by promoting bifidobacteria and/or lactobacilli populations, and increased absorption of calcium and minerals [3]. Currently, most prebiotics developed are the non-digestible oligosaccharides i.e. fructo-oligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharides (IMO), soybean oligosaccharides (SOS), lactulose, lactosucrose except for polysaccharide-inulin [4]. Probiotics are beneficial bacteria particular bifidobacteria and lactobacilli. Probiotics have been demonstrated to impact on the immune function and such approaches may be useful in stimulation of the aging, dysregulated immune system bringing about reduced inflammatory responses, and balancing gut microbiota [5].

Prebiotic and probiotic researches in Thailand have been studied on several aspects such as alternative sources of prebiotic from fruits and vegetables in Thailand, and functionalities and properties of prebiotics to gut microbiota and gut health. The researches were focused on study the possibility of agricultural raw materials in Thailand to replace sources of commercial prebiotics. In the food industries, several oligosaccharides have received increasing attention as key components for functional foods and nutraceuticals products. Prebiotics are non-digestible oligosaccharides which have been shown to have properties that can modulate gastrointestinal problems and improve gut health and well-being. Recent researches much pay attention to find alternative sources, improve specific properties and proof on health benefits of these prebiotics.

MATERAILS AND METHODS

Wichienchot and colleagues [6] were studied on several plants as source of prebiotic in Thailand and studied on optimal conditions for extraction of oligosaccharides from plants. Thirteen types of fruits and vegetables commonly grown and consumed in southern Thailand were selected based on their physicochemical characteristics, which suggested the presence of prebiotics or soluble dietary fiber, abundance, low cost, and the amount of waste they generated that could be utilized. Table 1 lists the plants and their parts used in the studies. Samples were analyzed for moisture content, extract yield, and indigestible polysaccharide concentrations were investigated.

Table 1 Plants and their various parts used in the analysis for prebiotics.

Plant	Part used in the studies
Banana (*Musa sapientum* Linn.; Thai name: Kluay Naam Wah), a. mature green, b. ripe	a. Skin b. Flesh
Okra (*Hibiscus esculentus* Linn.; Thai name: Krajiab Kheaw), mature green	a. Pod
Jackfruit (*Artocarpus heterophyllus* Lam.; Thai name: Ka Nhoon), ripe	a. Skin (inner rind, i.e. excluding the outer skin) b. Flesh c. Seed
Germinated brown rice (*Oryza sativa* Linn.; Thai name: Kao Ngog)	a. Germinated grain
Rambutan (*Nephelium lappaceum* Linn.; Thai name: Ngoh), ripe	a. Peel b. Flesh c. Seed
Durian (*Durio zibethinus* Linn.; Thai name: Turian), ripe	a. Shell
Jampadah (*Artocarpus integer* Merr.), ripe	a. Skin b. Flesh c. Seed
Huasa potato (*Coleus parvifolius* Benth; Thai name: Man Kee Nhoo), mature	a. Tuber
Tamarind (*Tamarindus indica* Linn.; Thai name: Ma Kham), ripe	a. Flesh b. Seed coat

	Plant	Part used in the studies
		c. Seed cotyledon
0	Coconut (*Cocos nucifera* Linn.; Thai name: Ma Prao), young fruit	a. Flesh
1	Mango (*Mangifera indica* Linn.; Thai name: Ma Muang), mature green	a. Skin b. Flesh Seed
2	Fan palm fruit (*Borassus flabellifer* Linn.; Thai name: Luke Taan)	a. Pericarp of ripe fruit b. Flesh from young fruit c. Embryo
3	Dioscorea tuber (*Dioscorea membranacea* Pierre; Thai name: Hua Khao Yen)	b. Tuber

Galactooligosaccharide (GOS) was produced according to the study of Hemmaratchirakul and co-workers [7]. The β-galactosidase was partially purified from *Lactobacillus pentosus* var. *plantarum* BFP32. This strain was isolated from fermented pork sausage that could produce β-galactosidase. The enzymatic reaction was carried out by mixing β-galactosidase with lactose or milk in batch and UF membrane reactor. Effect of reaction pH and temperatures for production of GOS were investigated. The method for purification of GOS was done using diafiltration technique in nanofiltration membrane. Optimal conditions for production and purification of GOS have been investigated. Chemical composition of GOS was determined by HPLC. Prebiotic property of the GOS was studied in batch culture system. Prebiotic index (PI) was calculated according to the changes of bifidobacteria, lactobacilli, bacteroides, clostridia and eubacteria (total bacteria) by fluorescence *in situ* hybridization (FISH) technique.

Fructooligosaccharide (FOS) was produced by study of Prakobpran *et al*. [8]. Fructosyltranferase (FTase) was extracted from tuber of Jerusalem artichoke (*Helianthus tuberosus* L.). The enzyme was further purified by two chromatographic steps (anion exchange and affinity chromatography). The FTase was studied on kinetics for FOS synthesis using sucrose as substrate. Optimal conditions for production of FOS were investigated. Chemical compositions of FOS was analyzed by HPLC. Purification of FOS was successful by yeast fermentation to remove residual sugars. Prebiotic property of FOS was studied in batch culture by fecal fermentation. Prebiotic index (PI) was calculated according to the population changes of 5 bacterial genus by fluorescence *in situ* hybridization (FISH) technique.

Four formulas of tuna products added inulin were developed together with Tropical Canning (Thailand) Public Company Limited, Songkhla, Thailand. These products were canned tuna in spring water and tuna in mayonnaise and pouched tuna in salad cream and tuna in thousand island cream. Addition of inulin at 3, 5, 7 and 10%, w/w was studied. The effects of the addition of a prebiotic (inulin, Orafti®-HP) on the physical properties and sensory properties of these products were studied. The canned tuna in spring water and pouched tuna in salad cream were fermented in *in vitro* fecal fermentation with three-stage continuous system [9]. The efficacy of tuna products added 5% inulin were evaluated on their benefits to gut health by 30 volunteers consumption for 2 weeks. Feces was taken to enumerate bacterial by FISH technique and questionnaire was asked for type and odor of faces, mass of faces and frequency of defecation.

Xylooligosaccharides (XOS) was produced from rubber wood sawdust. Rubber wood sawdust was sieved through sieve No. 20 MESH to obtain small size then it was soaked in water. The extraction of xylan from rubber wood sawdust had two methods; alkaline extraction, and acid extraction. Xylans were further hydrolyzed by xylanase to obtain XOS. Optimal conditions for production of XOS were investigated. The chemical compositions of XOS were determined by HPLC and molecular weight distribution was analyzed by HPSEC [10].

β-glucans from eight edible mushrooms (*Schizophylum commune* Fr, *Auricularia auricula* Judae, *Pleurotus osttreatus*, *Pleurotus sajor-caju*, *Flammulina velutipes*, *Lentinus edodes*, *Volvariella vovacea* and *Boletus griseipurpureus*) were investigated. Dried mushroom powder was pre-treated with cellulase. The pre-treated mushroom powder was further extracted in autoclave at temperature of 130 °C for 45 and 90 minutes to obtain β-glucan. Determination of β-glucan content in extractable solid of mushroom samples was done by a mushroom β-glucan kit. Cost of mushroom per β-glucan content, yield and β-glucan content of eight mushrooms were compared [11].

RESULTS

Among the 30 parts of 14 plants, six appear to have the highest potential for commercialization based on extract yield and the amount and type of indigestible oligo-polysaccharides. These include dragon fruit, palm flesh, palm embryo, jackfruit flesh, jackfruit seed, and okra pod. At least three of them, dragon fruit, jackfruit flesh and seed, were confirmed on their prebiotic property by selectively *in vitro* colonic microflora fermentation in an artificial colon system.

Table 2 Moisture content, extract yield, and indigestible oligo-polysaccharide concentrations in ten plant samples

Plant	Part	Moisture content (%wet weight±SD)	Solvent	Extract yield (%dry weight±SD)	Indigestible polysaccharides, acid/enzyme digestion (mg/g dry extract±SD)	Indigestible polysaccha-rides, H_2SO_4 digestion (oligosaccharides) (mg/g dry extract)
Palm fruit	Pericarp	85.53±0.10	EtOH 95%	51.69±0.40	705.80±3.83	14.13
Jackfruit	Skin	88.65±0.03	EtOH 95%	71.54±0.01	689.08±15.21	0.00
Jackfruit	Flesh	81.76±0.01	EtOH 95%	59.43±0.07	605.76±16.55	98.05
Rambutan	Flesh	82.81±0.07	EtOH 50%	55.73±2.65	566.83±8.42	9.43
Jampadah	Flesh	69.83±0.06	EtOH 95%	34.11±0.12	542.56±13.82	2.40
Young	Flesh	86.45±0.06	CW	22.66±5.59	513.87±4.29	0.00
coconut	Pod	89.99±0.03	EtOH 50%	12.39±0.01	460.73±17.05	49.15
Okra	Embryo	77.66±0.20	EtOH 50%	26.54±0.96	409.85±2.88	33.69
Palm fruit	Seed	71.50±0.20	EtOH 50%	16.00±0.08	403.44±5.63	29.35
Jackfruit	Flesh	91.99±0.04	EtOH 95%	44.94±0.26	334.87±19.45	47.20
Palm fruit						

SD = standard deviation; EtOH = ethanol, CW = water at ambient temperature. [a] SD was calculated from averaged SDs of total sugars and reducing sugars. [b] Due to the calculation method used it was not practical to calculate SD.

Among 52 lactic acid bacteria (LAB) isolates for production of GOS, BFP32 showed highest intracellular β-galactosidase activity and GOS yield. It was identified as *Lactobacillus pentosus* var. *plantarum* by 16S rDNA sequencing. The optimal pH and temperature for synthesis of GOS were 7 and 50°C, respectively (Figure 1). Composition of GOS consisted of oligosaccharides with having DP of 3, 4 and 5. A mixture of GOS was purified successful by sequential bacterial and yeast fermentation whereas nano-filtration could be used for partial purification.

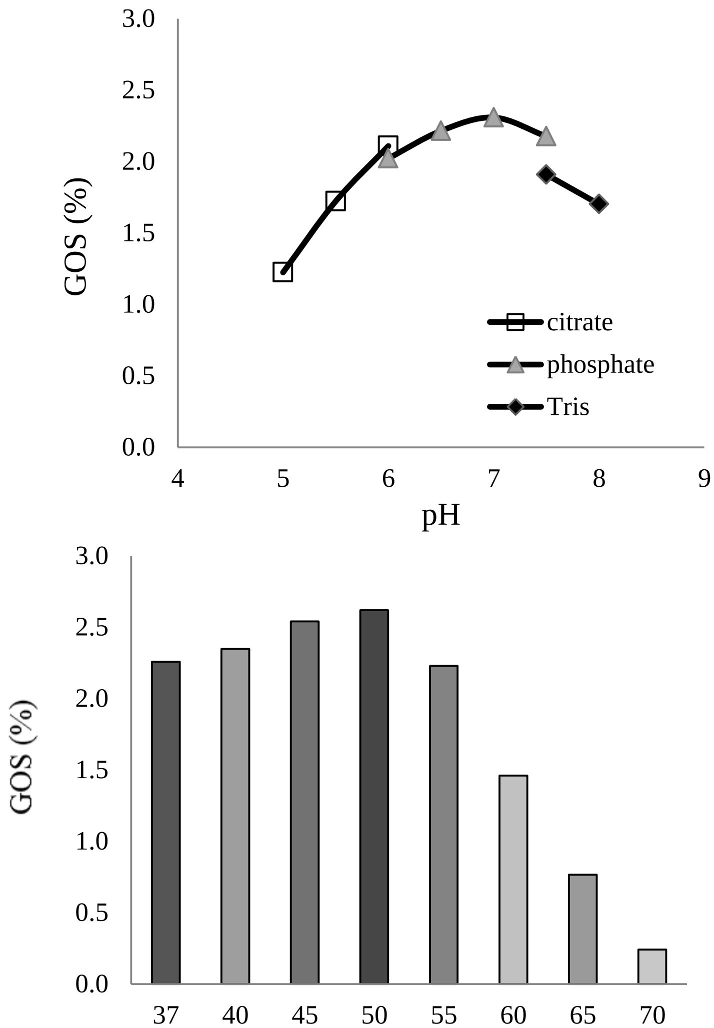


Figure 1. Production of galactooligosaccharide using β-galactosidase (100 U/ml) at 50 °C and pH 7.0 with 60% lactose as substrate; (■) GOS; (▲) lactose; (✕) glucose; (◆) galactose.

Fermentation of GOS in fecal batch culture for evaluation of prebiotic property was performed. The results showed that GOS produced in the study and commercial GOS could increase lactobacillus and bifidobacteria significantly ($P<0.05$) (Figure 2). Prebiotic properties can be calculated from prebiotic index using numbers of cell counts by fluorescent *in situ* hybridization (FISH) technique. The population changes in numbers of lactobacilli, bifidobacteria, clostridia, bacteroides and eubacteria were calculated. The result showed positive prebiotic effects both GOS produced in the study and commercial GOS. GOS produced in the study has prebiotic index of1.19, and commercial GOS has prebiotic index of 1.68.Conversely, mixed sugars showed negative prebiotic effect (PI = -0.01) due to mixed sugars composed sugars that it was metabolized by mostly harmful fecal bacteria.

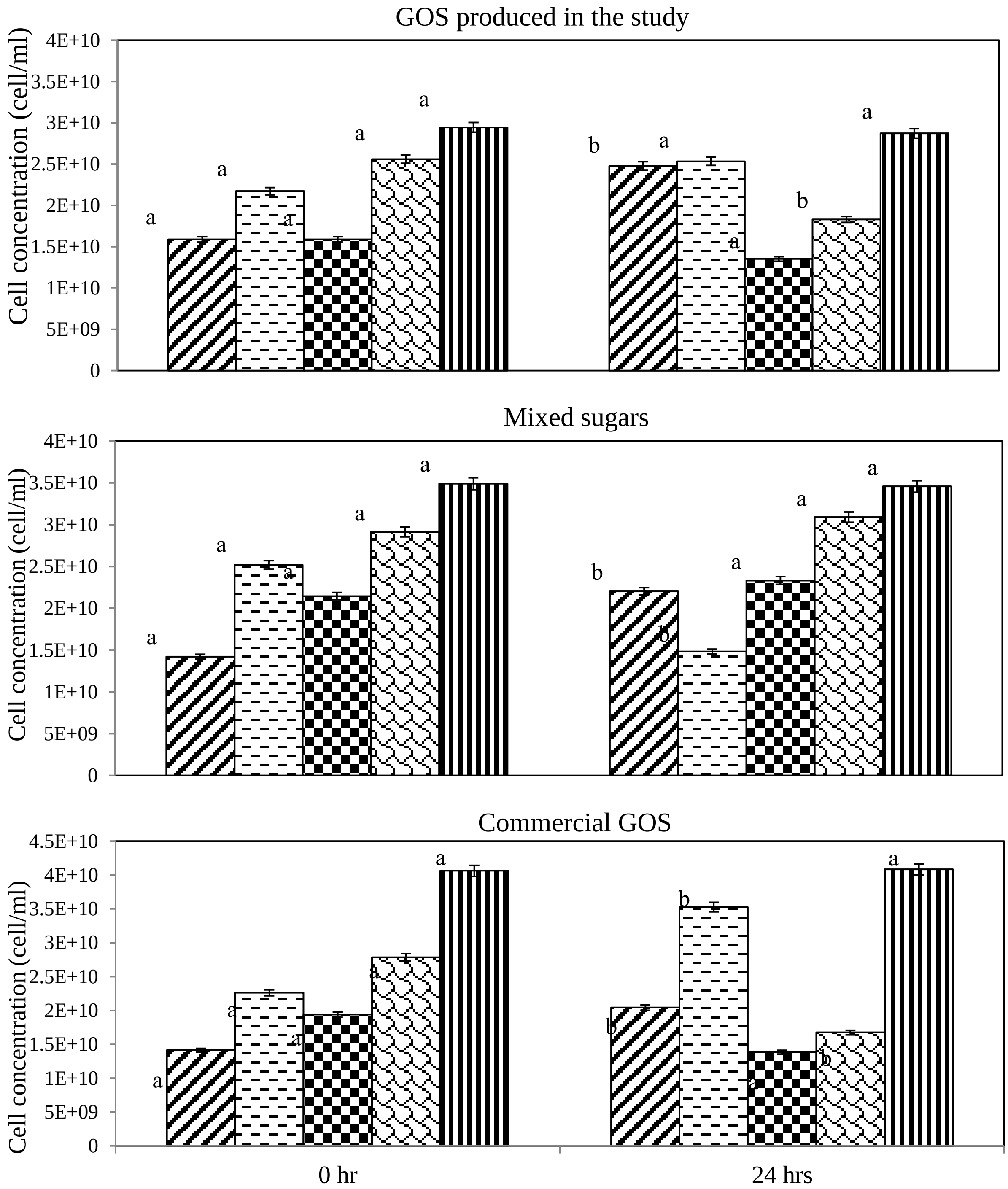
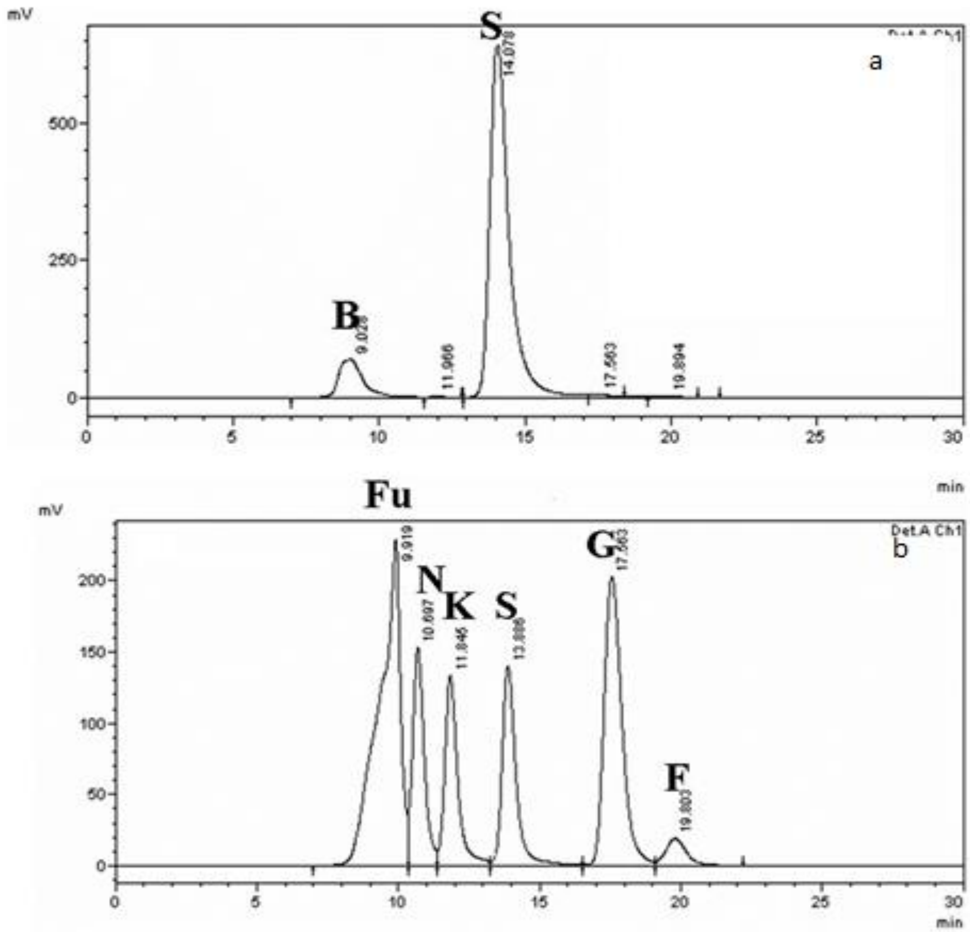


Figure 2. Cell concentrations in batch culture of GOS produced on the study, mixed sugars, and commercial GOS as carbon source at 0 and 24 hours; () Lactobacillus,() Bifidobacteria,() Bacteroides, () Clostridia and Eubacteria ()

Different letters is statistically significant ($p<0.05$)

A crude extract from tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) had transfructosylating activity for biosynthesis of FOS from sucrose. Optimal conditions for production of FOS were 0.26 U FTase, incubated with 0.46 M sucrose as substrate at pH 5.4 and 35ºC for 144 h. A maximum yield of scFOS (DP≤5) was obtained (54.46%) (Figure 3).



B: 0.1 M K phosphate buffer; S: Sucrose, F: Fructose; G: Glucose; S: Sucrose; K: 1-kestose (GF_2); N: nystose (GF_3); Fu: 1-β-fructofuranosylnystose (GF_4)

Figure 3. Production of FOS by enzymatic synthesis of 0.26 U FTase from 105-120 days old tubers of tropical Jerusalem artichoke and 0.46 M sucrose as substrate, pH 5.4 at 35ºC for 0 (a) and 144 h (b).

Fermentation of FOS in fecal batch culture system for evaluation of prebiotic property was tested. The result showed that FOS produced in the study and commercial FOS were significantly ($P<0.05$) increased numbers of lactobacilli, bifidobacteria and decreased number of clostridia groups (Figure 4). Meanwhile mixed sugar was significantly ($p<0.05$) increased bifidobacteria, clostridia but decrease lactobacilli.

Prebiotic properties were evaluated from prebiotic index. The microbial group that counted as lactobacilli, bifidobacteria, clostridia, bacteroides and eubacteria. The result showed positive value both FOS produced in the study and commercial FOS. FOS produced in the study and commercial FOS had prebiotic index of 1.29 and 1.14, respective. However, mixed sugars showed negative prebiotic effect with PI of -1.9.

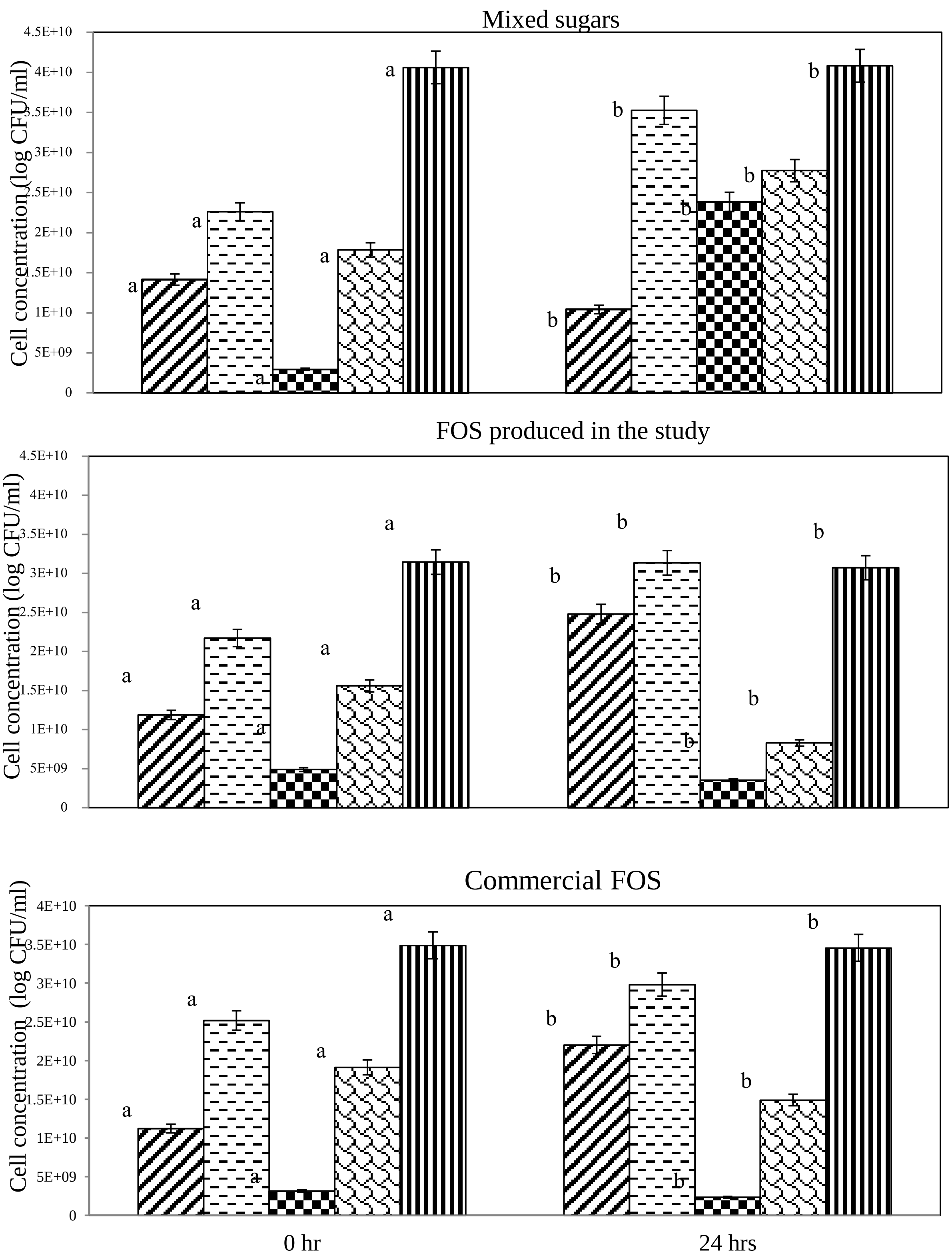


Figure 4. Cell concentration in batch culture using mixed sugar, produced FOS and commercial FOS as carbon sourceat0 and24 hours; ( ) Lactobacillus, ( ) Bifidobacteria, ( ) Bacteroides, ( ) Clostridia and ( ) Eubacteri. *Different letters is statistically significant* (p<0.05)

Four formulas of canned tuna in spring water and tuna in mayonnaise and pouched tuna in salad cream and tuna in thousand island cream with added inulin were developed for commercial production. An addition of 5 % inulin for tuna in spring water and 7 % inulin for tuna in mayonnaise, tuna in thousand island and tuna in salad cream are recommended. In clinical study of tuna in spring water and tuna in salad cream added 5% inulin, it was found that inulin addition helps on improve of bowel regularity. The changes in selected bacterial populations in three-stage continuous culture that presented prebiotic index are shown in Figures 5.

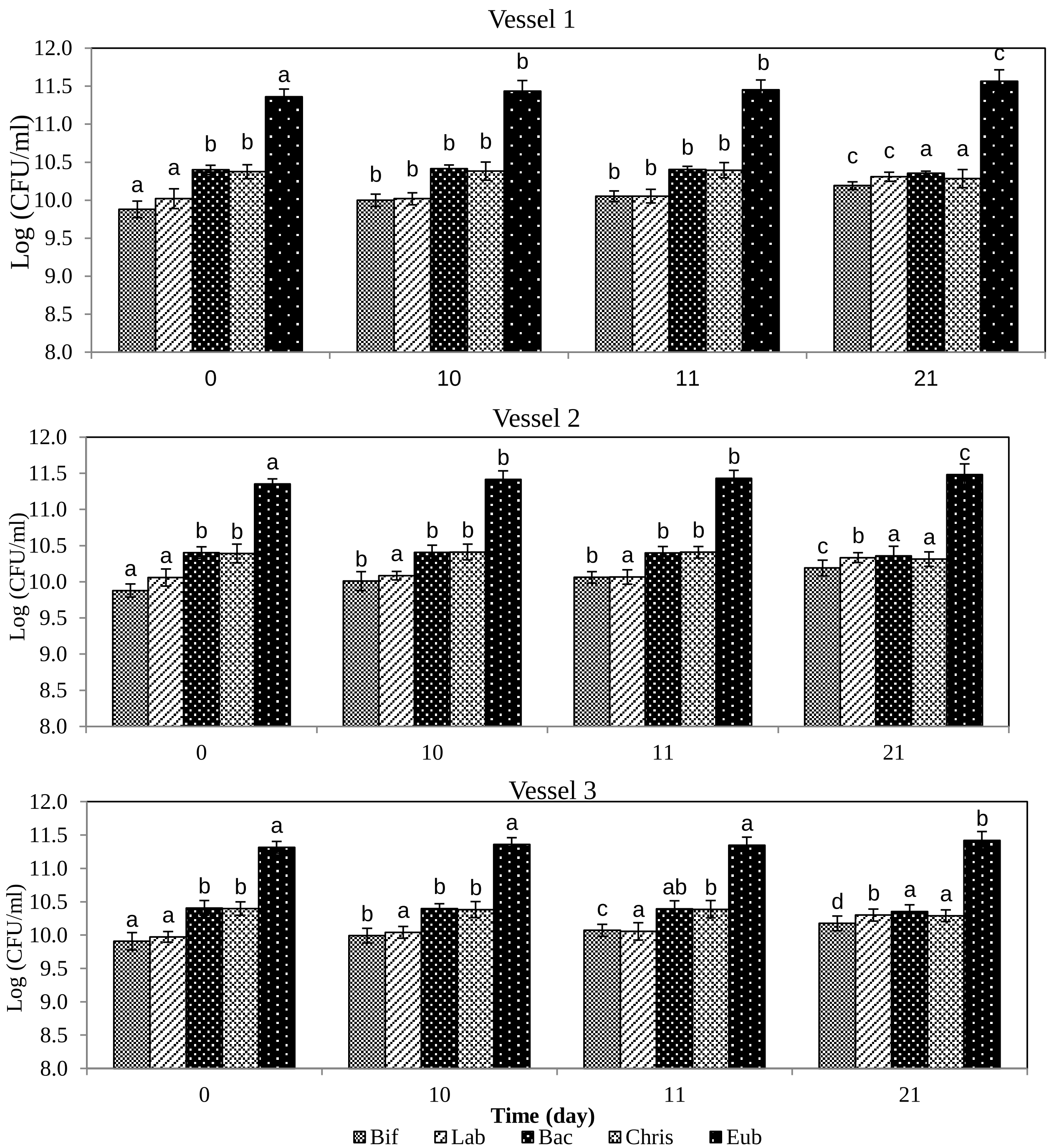


Figure 5. Numbers of fecal bacteria in three-stage continuous fermentation of tuna in spring water added 5% inulin. Values with different superscripts within the same bacterial species mean it has significantly difference ($p<0.05$).

The results showed that the highest prebiotic effect was found in tuna in spring water added 5% inulin. The prebiotic index (PI) of tuna in spring water added 5% inulin in vessel 1, 2 and 3 was 1.28, 1.63 and 1.82, respectively. The prebiotic index of tuna in salad cream added 5% inulin in vessels 1, 2 and 3 was 0.93, 0.73 and 0.77, respectively. The result gives a positive prebiotic index of both tuna products added 5% inulin. Thus addition of 5% inulin to standard formulae of tuna product resulted in significantly ($p<0.05$) inc rease of beneficial colonic bacteria.

By-product from rubber wood sawdust could be used as alternative source of xylo-oligosaccharide (XOS). The extract obtained by acetic acid extraction at various conditions. It was found that acetic extraction produced mostly XOS whereas alkaline extraction produced mostly xylan. XOS derived by acid extraction found only at high temperatures (150 and 200 °C) and longtime at least 1 hour. Optimal condition for extraction of the sawdust by acid under high temperature was 150-200 °C for 1-2 hours giving XOS with MWCO in the range of 8-11 KDa (Table 3).

Table 3. Molecular weight distribution of xylan and XOS rubber wood sawdust extracted by acetic acid

Treatment	Conc. of acid (%)	Temp. (^{o}C)	Time (min)	MW (Daltons)
1	0	100	60	-
2	0	150	0	-
3	0	150	120	8,034
4	2.5	200	60	-
5	2.5	100	0	-
6	2.5	100	120	-
7	2.5	150	60	-
8	2.5	200	0	-
9	2.5	200	120	138.120
10	5	100	60	-
11	5	150	0	-
12	5	150	120	11,465
13	5	200	60	9,045

Among eight edible 8 commercial mushrooms, *Schizophyllum commune* had highest total β-glucan content (59.87% dry basis) and the lowest costof mushroom (0.06 USD/g β-glucan). The secondorder was *Auricularia auricula* Judae had β-glucan of 31.51% (of total glucan) and the cost of mushroom was 0.07 USD/g β-glucan as shown in Table 4.

Table 4. Summary of cost of mushroom, yield and β-glucan content and cost/β-glucancontent of eight mushrooms

Name	Cost of mushroom (USD/kg)	Yield (g/kg dry wt.)	β-glucan (% dry wt.)	β-glucan content (g/kg fresh wt.)	Cost/β-glucan content (USD/g)
Schizophylum commune Fr	6.06	134.69	59.87	80.65	0.06
Auricularia auricula Judae	1.82	79.99	31.51	25.20	0.07
Pleurotus sajor-caju (Fr) Sing	3.03	101.13	37.61	38.04	0.08
Pleurotus osttreatus (Fr) Kummer	6.06	104.33	32.93	34.36	0.18
Volvariella vovacea (Bull, Ex, Fr) Sing	4.85	107.40	27.85	29.91	0.16
Lentinus edodes (Berk) Sing	10.61	145.58	25.50	37.12	0.29
Flammulina velutipes (Curt, Ex, Fr) Sing	4.55	84.35	19.37	16.34	0.28
Boletus griseipurpureus Corner	6.06	60.50	12.09	7.31	0.83

DISCUSSION

Prebiotic differs from the classical dietary fibers specifically soluble dietary fibers which selectively stimulates the growth and/or activity of beneficial bacteria, i.e. bifidobacteria and lactobacilli species [12]. Recent research works on prebiotics in Thailand including screening of prebiotics from natural occurring in fruits and vegetables. However, cheaper samples such as wastes from juice industry, vegetable processing factory and agricultural by-products are more interested because it has low cost for production. Raw materials used for industrial production of inulin are chicory and artichoke. Roots of these plants contained about 60% and 25% (dry wt.) inulin, respectively [13]. However, the maximum content of oligosaccharides found in our researches was less than 10% i.e. dragon fruit contained 5-8% FOS [14]. Alternative way to obtain high content of oligosaccharides might be succeeded by enzymatic synthesis approach such as GOS and FOS production from novel enzyme with high productivity and more specificity. A novel source of β-galactosidase in our study can be applied for production of low lactose milk with high GOS by a single step and this process has been patented. Plant tissue culture is also a new approach for production of FOS. XOS is one of emerging prebiotic due to it has high prebiotic effect even at low dose and commercial XOS is made from corncobs [15]. Any raw material suitable for production of XOS have to contain high content of xylans. Currently, commercial β-glucans have been produced either from oat and barley or brewery's yeast [16]. However, our recent data in rat study on immune stimulation showed that β-glucans from mushroom has high effective comparable to yeastβ-glucans. Today consumers are concerning for nutraceuticals and functional foods containing active ingredients [17]. Prebiotics are more widely used in foods particular in dairy products. Furthermore, health claim of product containing prebiotic need to be clinically proven.

CONCLUSIONS

Prebiotic differs from the classical dietary fibers specifically soluble dietary fibers which selectively stimulates the growth and/or activity of beneficial bacteria, i.e. bifidobacteria and lactobacilli species. Recent research works on prebiotics in Thailand including oligosaccharides from natural occurring in fruits and vegetables, oligosaccharides from enzymatic synthesis such as galactooligosaccharide (GOS) and short chain fructooligosaccharide (scFOS). Xylooligosaccharide (XOS) from rubber wood sawdust and β-glucan from edible mushrooms. Application of inulin in tuna products have been clinically proven on gut health function.

List of Abbreviations: LAB, lactic acid bacteria; GOS, galactooligosaccharide; scFOS, short-chain fructooligosaccharide; XOS, xylooligosaccharide; IMO, isomaltooligosaccharide; SOS, soybean oligosaccharide; SCFA, short-chain fatty acid; PI, prebiotic index; FTase, fructosyltransferase; FISH, fluorescence *in situ* hybridization; DP, degree of polymerization; MWCO, molecular weight cut-off; HPSEC, high performance size exclusion chromatography

Competing Interests: The authors declare that they have no conflicts of interest.

Author's Contributions: All authors contributed to these studies.

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